

Jul 24, 2024

🌐 How to Improve the Reliability of qPCR Detection with Target Gene-optimized Internal Standards

DOI

<https://dx.doi.org/10.17504/protocols.io.14egn6e7pl5d/v1>

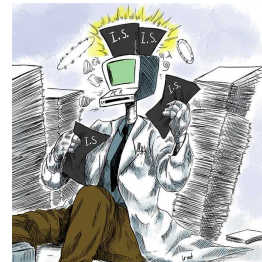
Jonathan Phillips¹, Gregor Blaha¹

¹University of California, Riverside



Gregor Blaha

University of California, Riverside



Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.14egn6e7pl5d/v1>

Protocol Citation: Jonathan Phillips, Gregor Blaha 2024. How to Improve the Reliability of qPCR Detection with Target Gene-optimized Internal Standards. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.14egn6e7pl5d/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: July 21, 2024

Last Modified: July 24, 2024

Protocol Integer ID: 103799

Keywords: qPCR, Internal Standard, amplicon-specific, ddPCR, qpcr detection, pathogen detection in field sample, pathogen detection, reliability of qpcr detection, infected citrus sample, achieving robust qpcr result, accurate detection of target gene, robust qpcr result, detecting cla, pathogen signal, qpcr, pathogen, citrus sample, absence of the pathogen, clas in hlb, target gene, accurate detection, detection, inhibitors present in the sample

Funders Acknowledgements:

California Department of Food and Agriculture

Grant ID: 21-0001-056-SF

Abstract

Achieving robust qPCR results requires both sensitive and accurate detection of target genes. This is particularly challenging for pathogen detection in field samples because the absence of a detection signal does not necessarily indicate the absence of the pathogen. Inhibitors present in the sample can suppress the pathogen signal, leading to false-negative results. The presented protocol details the use of an optimized internal standard for detecting CLAs in HLB-infected citrus samples.

Attachments



[05_target-gene-optim...](#)

380KB

Image Attribution

For permission to use the image or to contact the creator of the image, irod, please contact the lead author at gregor.blaha@ucr.edu



Protocol references

Anonymous (2010) ISO 4787.2010 Laboratory Glassware – Volumetric Instruments – Methods for Testing of Capacity and for Use.

Anonymous (2012) ASTM E969-02: Standard Specification for Glass Volumetric (Transfer) Pipets

Anonymous (2022) ASTM E542-22: Standard Practice for Gravimetric Calibration of Laboratory Volumetric Instruments

Maar D., et al. (2020) Transitioning Your Assay from Quantitative PCR to Droplet Digital PCR. Bio-Rad bulletin 7320.

Huggett J.F., et al.(2008) Differential susceptibility of PCR reactions to inhibitors: an important and unrecognised phenomenon. BMC Res Notes, 1, 70. PMID: 18755023. doi: 10.1186/1756-0500-1-70.